Nutech O_3, Inc.

2540 South Walter Reed Drive
Suite D
Arlington, VA 22206

NOAA Cooperative Agreement Program:

A Collaborative Ballast Water Treatment Test Bed Platform for the Purpose of Determining the Effectiveness of Injecting Ozone Into Ballast Water to Kill Invasive Aquatic Species

Grant Number: NA04OAR4170149

Response to Scientific Advisory Panel Comments on Scientific Protocol

Submitted April 28, 2005

1) Summary of Meeting of Advisory Group:

At this meeting, general comments and issues regarding the Nutech-O3 draft experimental proposal were discussed, focusing on the need for a more explicit experimental protocol that fully outlined the state of existing knowledge, particular at the lab and intermediate scale, demonstrated the need for ship-board testing, and addressed engineering concerns of effectiveness and safety on-board ship.

In particular the following concerns were raised:

- Does the proposed design work to get ozone into solution? Work that has demonstrated the effectiveness and efficacy of this system should be specifically referenced within this document.
- While the proposal is a good conceptual outline, it lacks the specificity needed for an experimental protocol.
- The protocol lacks a component to evaluate "whole ship" considerations, i.e. concerns over corrosion impacts, ozone leakage, safety, etc.

2) General Comments

- Ozone is known to have deleterious effects on non-metallics that are often associated with seawater systems, including valve seals and flange gaskets. This same concern should apply to ballast tank coatings. What efforts are being made to quantify these effects?
- The Protocol does not describe the actual experiments to be conducted.

The Protocol does not include a discussion of how the experiments will allow the researchers to reach their objectives.

- This document is an adequate conceptual outline of the proposed work. I do not consider it to be a formal "experimental design". There is far too little detail about how controls and treatments will be managed, sampled, and analyzed.
- I think the project team should lay out the current understanding of the system, the underlying treatment mechanism, and the potential environmental effects of the residuals, based on work to date. Then specific hypotheses could be framed and experiments to test these hypotheses designed. The need for specific experiments should be clearly articulated.
- The project should be phased, with clear "gates" established between substantively different phases. The injector should be well tested for its ability to deliver adequate amounts of ozone to achieve design TRO levels at intermediate scale before a shipboard test is permitted. Design TRO levels should be well established in the lab before any ship tests. WET tests should be done at small scale before any tests in the environment. This proposal is explicitly titled as a shipboard project – but much of the necessary work that remains to be done is best done in the lab. Will this preliminary work be completed before the expense of the shipboard work is committed?

3) Specific Comments (Comments on the Scientific Proposal are inserted into the text in italicized blue font.)

Comment: (1) We are preparing several papers that analyze our research from 2000 through 2005
1. Dr. Russell Herwig our senior biologist, from the University of Washington, is in charge of preparing the Report on the experimental studies conducted both on board the Tonsina and at the University of Washington's Marrowstone test facility.
2. Dr. William Cooper and Dr. Hans van Leeuwen, our principal scientific investigator and scientific advisor, are performing final review on a TRO – decay study.
3. Parametrix is preparing an Ecotoxicology paper from the first set of experiments on the Tonsina.
4. Dr. Herwig and Jake Perrins, of University of Washington, are preparing a Flow Cytometry analysis of the bacterial response to ozone involv … [1]

Comment: (2)Ozone injection by venturi is utilized in many drinking water treatment plants. Its effectiveness has been proven throughout the industry for many years. The focus of this research is to demonstrate the effectiveness o … [2]

Comment: (3) We agree, and note that these issues are covered as part of the design process, not the experimental protocol. Development of a detailed experimental design has not been presented before approval of the … [3]

Comment: (4)We agree and have covered these issues in design process. Prior to the ship owner allowing us to outfit the ship with this equipment, "whole ship" considerations will be covered as part of a detailed Hazar … [4]

Comment: (5)We have conducted extensive corrosion studies at the LaQue Corrosion Institute. See study at (http://www.nutech-o3.com/files/laque_corrosion.pdf). In addition, it should be pointed out t … [5]

Comment: (6)See Comment #3.

Comment: (7) We were looking to this being an iterative process and the formal design will be completed once the Panel approves the general approach. Our research will focus on the absolute number of organisms remaining in … [6]

Comment: (8)See comment #3.

Comment: (9)Extensive discussions on the understanding of the system were included in our grant proposal and can also be found in our final report on the Tonsina experiments. (http://www.nutech- … [7]

Comment: (10)This preliminary work has either been done or is being done and has led to a conservative design target TRO of 2.5 mg/l. Also see comment #1.

Nutech O3 Ballast Water Ozone Treatment Project on board the T/V Prince William Sound.

Experimental Design

I. Introduction

The introduction of non-native coastal species across the bio-geophysical barriers of the ocean by ships through the discharge of contaminated ballast water is of great ecological and economic concern. The introduction, for instance, of zebra and quagga mussels into the Great Lakes via the discharge of ships' ballast water has led to irreversible ecological damage, has had devastating economic consequences, and has led to secondary infestations into the Mississippi River and its tributaries. Similar discharges have destroyed commercially important shellfish populations in the Chesapeake Bay. Ship's ballast water exchanges at sea have been shown to be ineffective, and they are very difficult to police. Ballast water treatment is more reliable and compliance with any Coast Guard treatment requirements can be verified economically and in a short period of time. Among the possible treatment technologies, ozonation stands out as an environmental friendly technology. The ozone residual, although short-lived, is a powerful disinfectant, while its reaction with bromide in seawater results in a longer-lasting residual of hypobromous acid. The combined effects of ozone and hypobromous acid, as well as the super-saturation of the seawater with oxygen, have been shown to result in superior synergistic biocidal and minimal ecological effects. The resulting combined disinfectant residual is measured and expressed as the total residual oxidant (TRO). Much of this research program is focused on the biological and chemical effects of the TRO. The efficiency of the ozone transfer system and its operation using a full-scale system installed aboard the oil tanker T/V Prince William Sound will be tested while the ship is sailing under normal operating conditions.

- *The introduction purports that ballast water exchange is ineffective. My understanding was that that some experiments had shown that there was up to 98% removal of organisms using ballast water exchange as a treatment.*
- *The highlighted sentences should include citations.*

II. Objectives

The main goal is to establish the treatment efficacy of the ozone process under normal ship operations with the view of preventing the transfer and release of aquatic nuisance species. To achieve this goal, four [4] treatment objectives can be identified:

- *The terminology "normal ship operations" could be misleading, as each ship is unique. Therefore, "non-experimental ship operations" or some other terminology could better express the author's meaning.*

Treatment Objectives

• To achieve established limits, e.g. State of Washington and/or IMO Treaty Goals, or other criteria as developed.

- *I agree that the main goal is to establish the best treatment efficacy of the process. Therefore, the first bullet under the treatment objectives is unnecessarily limiting. We*

Comment: (11) The studies we already conducted on board the Tonsina proved that ballast water exchanges only remove approximately 64% of the invasive species found in a ship's ballast water. (see Final Report at http://www.nutech-o3.com/files/2002june15_finalreport.pdf).. Moreover, ballast water exchanges are only theoretically practical on ships using individual ballast water tanks that can be completely emptied before being refilled. Unless this procedure is followed, the contaminated ballast water will not be removed, it will only be diluted. This will be the case for most ships and certainly tankers, ensuring survival of large numbers of unwanted invasive species. That, in turn, could lead to regrowth of some these species to levels approximately equaling those prior to the ballast water exchange.

Comment: (12) Please identify the highlighted sentences.

Comment: (13) The term "normal" refers to the regular operations of the Prince William Sound. Those operations involve the transportation of Trans Alaska Pipeline crude oil, from Valdez, Alaska to refineries near Seattle, Washington, San Francisco, California and Long Beach, California. Further, "normal" is meant to imply that the process must not be so onerous as to modify or interrupt the "normal" operations of the vessel.

should not be trying to determine compliance with established limits whether it be the State of Washington or IMO. We should be designing a test that determines just how effective the process can be....period. We most definitely should not be using the State of Washington criteria since this is a federally funded project which should at least be using as a baseline the IMO criteria, but more likely should consider the possibility that the US standard will be more stringent (IMO's 10 organisms per unit volume above and below 50 microns versus the possibility of the US establishing a 1 or 0.1 standard). Therefore, using the IMO criteria as a baseline is fine, but we really need to know just how effective the process can be without regard to any criteria already established.

Comment: (14) The only scientifically meaningful way to analyze the effectiveness of this technology is to compare our on-board ship test results with established criteria, pending the passage of legislation and issuance of IMO rules in order to gauge our performance against these criteria. Ultimately, we will be determining how effective the treatment is without regard to any established criteria.

• To determine practical operating conditions and control guidelines

• To develop an understanding on the potential environmental effects of disposal of the treated ballast water

- *The third bullet is also too limiting in scope suggesting that we want to develop an "understanding" on the potential environmental effects of disposal of the ballast water. While I would hope that Nutech already has a pretty good understanding of this issue, it is obviously a necessary element of this project. However, a project that is being funded at over $1M should go further than just understanding this concept. It should include in its test protocols, all the necessary discussions and decision making processes inherent in state and/or federal permitting. At the end of this test, we hardly want to find we have a process that works on the ballast water critters but can't be discharged!!! This has to be included somewhere in the program otherwise it is valueless to the industry in real world application.*

Comment: (15)We will be developing data that relates to the photo degradation of the TRO and data that addresses the LC50 of TRO for different fish and waters. Our goal is to maximize kill rate and minimize toxicity of the discharge. The test data will be offered, when appropriate, to all federal and state regulatory agencies having an interest in this issue.

We have extensively researched the fate and degradation of TRO and will have a published report on these data shortly. However, more testing on this sensitive and complex aspect still needs to be done. Based on our research, we have determined that discharging ballast water carried in a ballast tank for several days, does not pose an environmental threat to the receiving water because most undesirable residuals will have expired prior to the treated water being discharged or immediately upon dilution and exposure to new reducing substances. Nevertheless, if any residual TRO is deemed to be at too high a level, it can be easily removed from the treated water by the addition of reducing agents such as various sulfide or sulfite compounds. This technique is already in common use in municipal waste water treatment facilities as a means of removing excess chlorine from disinfected effluents prior to discharge.

Additionally, the state and federal permitting effort, is beyond the scope of this grant.

• To obtain design criteria for shipboard ozonation during intake ozonation using side stream ozonation with back-mixing into the main flow and further contact in the ballast tanks

III. Tasks

To accomplish the objectives, this study is divided into four [4] areas as described below.

A. Total residual oxidant (TRO) measurement

1. To determine the rate of decay of TRO in ballast waters at full scale in several different (ports of call) waters from the Pacific Coast region.

- *Why does this need to be done shipboard? Is there some reason to think that the decay rate in full-scale tanks will be different than in a smaller tank in the lab? What water will be used, and how will the chemical compositions of the different source waters differ? This should be thought out, and a range of water conditions established that covers a wide range of global port conditions – otherwise it is just a test of decay in some randomly sampled water.*

Comment: (16)It is impossible to replicate the conditions of an 860 ft oil tanker carrying over 12 million gallons of ballast water in a lab. Ships have very complex hydraulic flow patterns in the ballast tanks, dead zones , relatively large depths, and opportunities to collect sediment, all very difficult to simulate. We are currently conducting decay rate experiments of water from different ports in the US to determine how they may differ from the ports of call of our test ship. The above notwithstanding, Congress directed that the $1.7 million set aside be used to test this technology on board a ship and not in a laboratory. See Conference Report 108-401 to accompany H.R. 2673, at page 596.

2. To determine the photochemical decomposition rate of TRO in seawater, to include experiments with different concentrations of TRO, various water qualities and various light conditions.

- *How is this different than #1, above? Is the former in the dark?*

Comment: (17)The ballast tanks are not exposed to light. The photochemical decay data is needed to establish dissipation of TRO in receiving ports if deballasting is done during daylight hours.

3. To confirm that TRO is an acceptable control parameter for ozone treated ballast water for ocean-going vessels.

- *How will this be "confirmed"? What are the underlying basic characteristics of an acceptable control parameter, and how will these aspects be evaluated?*

Comment: (18) We will use the TRO with time measurements to satisfy C x T values –TRO concentration times time - required, the approach followed in the Surface Water Treatment Rule in the Safe Drinking Water Act. It is our working hypothesis that the TRO will decay quickly enough so that the treated water will be safe to discharge yet it will remain in solution long enough to properly treat the ballast water to any proposed treatment standard.

4. As a subset of the TRO experiments, to relate oxidant measurements to changes in CDOM (chromophoric dissolved organic matter) using excitation emission matrix fluorescence spectroscopy (EEM or 3-D fluorescent spectroscopy). This will extend our database for the use of CDOM characterization as a potential monitoring approach.

- *None of the components in Task "A" seem to require, or be appropriate for, shipboard tests. In fact, I would expect to see careful lab and intermediate scale experiments first. These would then lead to specific hypotheses to be tested at full scale, if necessary.*
- *A. Total residual oxidant (TRO) measurement. It appears that there are too many variables to draw sound conclusions based on experimental results.*

Comment: (19)We are in the process of completing or have already completed extensive lab work that supports TRO x time as the best measure. See comment #1 and comment #18.

Comment: (20)The measurement of TRO is not complex, but its decay behavior is very much a function of water quality. We are in the process of developing models to be able to address this issue. However, we are confident that we have sufficient data to already be able to design our experiments for maintaining an adequate residual.

B. Biological effectiveness of ozone for ballast water treatment

1. To conduct a number of experiments at full scale to assess the efficacy of ozone treatment of ballast water by determining the concentration of target organisms at several trophic levels. These studies will be guided by the results of our past studies on the T/V Tonsina as well as emerging IMO and other standards.

- *Can the efficacy of the system (the new system, with injector) be demonstrated at intermediate scale under well controlled conditions? It is inappropriate to test this on a ship before it has been demonstrated at smaller scale, over a range of controlled state variables.*

Comment: (21)Ozone injection by venturi is an already proven technology and is widely accepted by the water treatment industry. We are testing the effectiveness of this technology full-scale on board a ship as instructed by Congress in House Report 108-401 accompanying HR 2673 at page 596.

2. Limited studies of targeted pathogenic organisms will be conducted under controlled laboratory studies. For example, Vibrio cholera or appropriate surrogates will be exposed to various concentrations of TRO and their treatability assessed.

- *Does "exposed to various concentrations of TRO" mean this will be done in a lab without the treatment process? If so, how will this be related to achieved conditions in intermediate or ship tests?*
- *B. Biological effectiveness of ozone ballast water treatment. The protocol should describe the results of past studies and standards that will guide experiments to assess the efficacy of ozone treatment – it would be good to see results that thoroughly demonstrate the efficacy of this approach at pre-shipboard scales.*

Comment: (22)No – we will ozonate water to obtain a TRO equivalent to that in treated ballast water and we will conduct these tests in the presence and absence of species that we can not use on full scale (Vibrio cholera, etc.).

Comment: (23)An extensive review of these past studies can be found in our Final Report on the Tonsina Experiments. (http://www.nutech-o3.com/files/2002june15_finalreport.pdf) Additionally, see comment #1.

- *It is unclear how determining the concentration of target organisms at several different trophic levels will lead to a determination of the efficacy of ozone treatment.*
- *B. Biological effectiveness of ozone ballast water treatment. The protocol should identify which organisms will be tested and under what limitations.*
- *Will this work lay the groundwork to establish the relationship (if any) between biological efficacy and more easily measured parameters such as TRO, CDOM, etc.?*

C. Effluent discharge testing:
1. To conduct laboratory studies using whole effluent testing (WET) guidelines for assessing the potential acute environmental toxicity of TRO.

2. To expand the number of species that are studied to determine chronic toxicity levels of TRO to evaluate the environmental safety of ozonated ballast water prior to discharge and to generalize the results.

D. Engineering and industrial health aspects of ozonation
1. To confirm the efficacy of ozone transfer using single point injection in a shipboard environment.

2. To determine the proper shipboard operating procedures for ozonation and ozone equipment.

IV. Considerations
1. The T/V Prince William Sound has a somewhat unique ballast system arrangement that we will use to our benefit during these experiments. Its ballast system uses two ballast pumps, the flow from which, during normal operations, is segregated from each other. One pump serves the forward-most set of ballast tanks, and the other pump serves the after ballast tanks.

2. In addition, a third, much smaller ballast pump services the vessel's aft peak and aft center salt water ballast tank.

3. This arrangement allows us to have maximum flexibility during our testing:
a. We intend to fit an ozone system designed to treat the output from one [1] main ballast pump. Due to installation issues on board the ship, this will likely be the pump that treats the after ballast tanks.
- *3.a. Previous sections of the protocol indicated that the ozone would be delivered to the tank through a side stream upon intake using a Venturi system. This section says that the researchers will fit an ozone system designed to treat the output of the main ballast pump. Will the ozone be delivered to the side stream or the main stream?*

b. Any of the forward ballast tanks could then be used as the experimental control tank, since that water would be totally isolated from the ozone treatment system.

- *How will the experimental design control for location effects (the control and treatment tanks will be at opposite ends of a very long ship)? While I don't know of*

Comment: (24)• Ballast water can contain organisms from viruses to living fish (in rare cases). Therefore, our initial studies on the S/T Tonsina looked at these various trophic levels to determine the effectiveness of ozone. Because we are working "in the dark" with respect to regulations, we feel that we need to test for and develop quantitative data on "kill" for as many target organisms as possible in ballast water.
• The new experiments will not include fish as it has been our position that if they are a real problem – then screening is the best alternative.

Comment: (25)See comment #3.

Comment: (26)Yes, this is our intent.

Comment: (27)Ozone will be injected into a side-stream with a venturi. The entire ozonated side stream flow will then be injected back into the main stream immediately. See the following diagram. http://www.nutech-o3.com/images/gallery/pages/side_stream.htm

any important differences, the whole point of a balanced design is to protect against unknown confounding factors. Ideally, treatment and control tanks would be in paired port and starboard tanks, and would alternate from side to side.

c. Finally, in the unlikely event that our ozone system cannot generate sufficient ozone to treat the main tanks, we could use the smaller aft peak system to inject into, and the treatment rate could then be more than five times what we can develop for the main tanks.

- *It seems like the designers should be able to determine apriori whether enough ozone could be "generated"…or does this mean that it is not known whether the injection method can get enough ozone into the flow to achieve the desired TRO? Has this been tested at one of the 1500 gpm test sites that have been used – U. Miami or Great Lakes Barge? If not, and if these sites are not available, an appropriate pump, pipe, and tank rig could be constructed for much less than the shipboard tests.*
- *3.c. If the ozone system cannot generate sufficient ozone to treat the main tank and the researchers use a smaller aft tank to inject into, would the researchers then test the aft tank or would they mix with the main tank?*

The goal will be to minimize the impact on the experimental vessel while still maximizing the number of experiments that are completed in the time we have available.

V. Experimental Considerations

A. Organisms to be studied – Trophic levels

a. Bacteria
b. Phytoplankton
c. Zooplankton
Microzooplankton < 100 µm
Mesozooplankton > 100 uµm < 1,000 µm
Megazooplankton > 1,000 µm (1 mm)

B. Control Studies

1. No ozone added to ballast water

- *Will the injector be used to inject air at the same pressure and with the same microbubble conditions? How will physical effects of the process be partitioned from the chemical?*

C. Time Course

1. TRO decomposition with time will be detailed for the ballast water on several voyages. This will then allow the use of concentration-time (CT) data as determined from other applications (such as water and wastewater treatment) and compared to ballast water treatment.

- *How will TRO be measured? What measures will be put in place to maintain TRO measurement calibration? What interval will these be measured on? To what resolution?*

Comment: (28)We agree that using paired tanks is an ideal set up because it makes the sampling much less labor intensive. There are no factors that make a port to starboard comparison any more scientifically balanced that a fore to aft comparison. Switching ozonation between tanks on different side of the ship would be a very expensive option for little gain in statistical significance. The above notwithstanding, the particular ballast piping configuration of this vessel precludes a port to starboard switch in ozone application without duplicating the whole system.

Comment: (29) Since this protocol was first published, we have completed further research which now makes us confident that we can reach our desired TRO. Therefore, the aft peak system will not be installed.

Comment: (30)Congress directed that the $1.7 million set aside be used to test this technology on board a ship and not in a laboratory. See Conference Report 108-401 to accompany H.R. 2673, at page 596.

Comment: (31)See comment #29.

Comment: (32)The purpose of this grant is "Determining the Effectiveness of Injecting Ozone into Ballast Water". The system being tested for effectiveness is one that injects ozone into ballast water. Our testing will not differentiate the physical effects from the chemical. There is no real need to differentiate any potential effect of micro-bubbles on disinfection or to expect any significant effect. Super saturation could cause embolisms in organisms, though, but any such minor effect would be evaluated as part of what is achieved with ozonation.

Comment: (33) TRO will be measured on ship trials using both sampling tubes that are distributed in the ballast tanks and if necessary with Niskin bottles. The analytical procedure is an EPA approved method for testing chlorine residuals – the DPD method and is used in conjunction with a Hach field test kit. We intend to conduct point sampling and measurements for the trials. Once we are sure that this is the control parameter we will purchase "off the shelf" instrumentation – similar to that used in water/wastewater facilities. Real time measurement of TRO will then be determined with built-in residual meters based on depolarization of a noble-metal electrode. The QA/QC will be part of the design and we anticipate using manufacture's recommendations. It should be pointed out that this is not a new measure and we will not be "breaking" any ground here – it is done every day in water treatment plants.

- *C. Time Course. Please specify how TRO decomposition with time will be detailed.*

Comment: (34)We anticipate sampling the treated ballast water using either our flow sampling lines or Niskin bottles over time from as early as possible – i.e. likely 1 – 2 hours after filling has begun through the length of the voyage – to Valdez. The data will be plotted and we will establish curves for different waters from different ports over the period of time covered by this grant. The continuous monitoring equipment will be computer linked and all data logged. This technology will be a very powerful tool for future compliance control.

2. From studies conducted both in the laboratory and previous testing aboard the T/V *Tonsina*, the treatment of bacteria, phytoplankton and zooplankton appears to be complete at the end of the ozonation of the ballast water. These studies used a diffuser technology that required that the ballast water tank be filled prior to ozonation. Then the ozone system was turned on and after 5 or 10 hours the target residual TRO was achieved. Samples were taken with time during ozonation and the efficacy was determined (kill rate).

3. The Venturi approach planned for these experiments will essentially provide TRO readings instantaneously and as the ballast tank is filled. Sampling during the fill process is not envisioned. Initial and focused studies will be conducted to sample immediately upon fill and subsequently with time to determine whether this approach is necessary on each ship-board experiment.

- *C. Time Course. Since the Venturi approach will deliver ozone through a side stream and then be mixed with the main stream through back-washing, how will the TRO readings be provided instantaneously?*

Comment: (35)We are not using backwashing, we are re-injecting the ozonated water into the mainstream. Readings will be taken downstream from the injection point before discharge into the tanks. It is at this point that we will have our instant reading. Once we complete the HazOp review and the final design level drawings are complete, we will have a precise location of the TRO meter(s).

D. WET Testing

An essential portion of this project is to access the potential toxicity of the ozonated ballast water. These studies will be conducted in the laboratory with water samples obtained from as many ports as possible, within budgetary constraints. At a minimum, mysid shrimp, topsmelt and sheepshead minnow will be studied for every water sample. Embryo-larval toxicity tests will also be performed. Where possible, additional organisms will be evaluated to extend our database.

- *The document generally is good but short on detail. Relating CDOM to the ozone studies and ballast water from various ports sounds excellent but again lacks detail. Other study items as well lack detail. The concepts are great but the plan needs to be expanded greatly to have sufficient detail for knowing that the study will generate the needed information. My vote is to endorse the general experimental concept but encourage more detail. The toxicity testing detail is especially important.*
- *D. WET Testing. It seems that the potential toxicity of ozonated ballast water should have been determined prior to shipboard testing and that the main goal of the shipboard test would be to determine if those levels of toxicity were achievable, both for treatment (high toxicity) and in the discharged water (low toxicity).*
- *D. WET Testing. Which additional organisms will be evaluated? What is the minimum number of organisms that will be evaluated to fulfill the objectives of the experiment?*

Comment: (36)These observations have already been addressed in some detail above.

Comment: (37)Indeed, such tests have been performed extensively and publications on the results are in preparation.

Comment: (38)See Experimental Design below. Sample size limitations prevent the study of sheepshead and topsmelt in all waters. These fish take much higher volumes of water to transport and our budget does not allow for the shipping of these large volumes of water.

The experimental design is shown in the Table:

Table 1. Experimental design for toxicity studies. (XX indicates the water and organism(s) that are being studied.			
Source Water	Organism Tested		
	Americamysis bahia Mysid Shrimp	*Cyprinodon variegatus* Sheepshead	*Atherinops affinis* Topsmelt
Artificial Sea Water	XX	XX	XX
Yuaquina Bay, OR	XX	XX	XX
Puget Sound, WA	XX		
Cape Fear River, NC	XX		
High DOC Impacted	XX		

E. Chemical Characterization of Water Quality

Extensive studies have been conducted under previous and on-going studies aboard the *T/V Tonsina*. Initial baseline data will be obtained for the different waters, defining the water quality.

1. Based on studies that have been published we have shown that little if any variation occurs with most of the normal water quality parameters. Minimal additional studies, if any, will be conducted during this project with respect to the normal water quality.

- *E. Chemical Characterization of Water Quality. 1. The first sentence should include a citation.*

Comment: (39)Please see our Final Report on the Tonsina experiments. (http://www.nutech-o3.com/files/2002june15_finalreport.pdf)

2. The dissolved oxygen (DO) concentration increases with increased ozonation. Experiments will be conducted to determine the increases in DO resulting from Venturi injection of ozone. Super-saturation with oxygen as a result of ozonation and pressure equalization in the ballast tank is expected to contribute to disinfection and help to maintain desirable aerobic conditions in the ballast tanks.

- *E. Chemical Characterization of Water Quality. 2. How does an increase in DO translate to super saturation?*
- *Increased DO should relate directly to increase corrosion rates particularly for localized and galvanic corrosion? What efforts will be made to quantify these effects.*

Comment: (40)During the first studies we were recording 20+ mg/L of O2; which by definition, is in the super saturation range.

Comment: (41)No, in fact an increased DO actually reduces corrosion. Information on corrosion rates can be found the study we conducted at LaQue Corrosion Institute. (http://www.nutech-o3.com/files/laque_corrosion.pdf)

3. The ozonation of seawater containing the bromide ion and organic material could theoretically result in the formation of bromoform and/or bromate ion as minor byproducts. In the studies that we have conducted thus far, we have never observed the formation of bromate ion. The formation of bromoform will be directly related to the total organic carbon (TOC) in the water. In our on-going studies, we have shown that although bromoform is observed, its concentration is well below any adverse effect level. Samples will be obtained from the different ports in this study and the bromoformformation rate will be determined. The ecotoxicity of these disinfection byproducts will be evaluated by the research team.

- *E. Chemical Characterization of Water Quality. 3. Is there a reasonable explanation for the relationship between the formation of bromoform and the total organic carbon in the water? Which organisms are being tested in on-going studies to determine that*

Comment: (42)Information on the relationship can be found in our full report on our Tonsina experiments. (http://www.nutech-o3.com/files/laque_corrosion.pdf)

the concentration of bromoform is well below the adverse effect level? Please define how this determination has been made. The researchers do not describe how the exotoxicity of byproducts will be evaluated. Will it be by WET?

- *None of these 3 components seem like they should be done at the shipboard scale. Have they been done at smaller scales?*

F. Open Ocean Exchange
There have been adequate studies that address this procedure and as the research and testing on viable technologies advances, the reliance on open ocean exchange is being questioned and lessened. Therefore, we will not include any open exchange experiments in this project. This also is in agreement with the idea of minimizing the impact on ship's operation.

- *Mid-ocean exchange is still the primary management practice required by current law. It is also likely that BWE will persist for a significant time as treatment standards are phased in for larger vessels. Without a better understanding of the relative effects of treatment and exchange, there is a danger of requiring vessels to practice management methods that may not be as effective. However, it is up to NOAA to decide whether a comparison is required.*
- *F. Open Ocean Exchange. It is unclear why the Scientific Protocol includes a section describing what the researchers will not do.*

G. Bacterial Indicator tests
Laboratory Experiments for selected pathogens and indicator organisms will be conducted to determine the efficacy of the ozone process in sea water. The organisms to be studied are:
a. Vibrio cholera
b. Escherichia coli
c. Enterococcus sp.

H. Ozone transfer studies
Ozone is a sparingly soluble gas resulting in less than complete dissolution. Ozone gas measurements, in gas samples after ozone contact and also that escaping from the ballast tank vents will be conducted. This will follow established methods used in the field of industrial hygiene. This will also determine the efficacy of ozone transfer and help establish design criteria for future applications.

VI. Sampling
Sampling access locations immediate prior to and following the ozone injection and multiple sampling lines located in the vertical and horizontal ballast tanks will be installed.
a. Sampling ports provided at the ballast water intake prior to ozone treatment, as well as at the point of ozone treatment, and immediately at the post-treatment point.

Comment: (43) The level of bromoform produced is quite low and there is no literature that suggests that bromoform is toxic to fish. Additionally, there are sources of bromoform in the marine environment by some algae. Therefore, we believe bromoform levels are well below adverse effect levels and will not conduct any test on bromoform during these studies.

Comment: (44)Yes.

Comment: (45)This grant will give us the opportunity to validate previous bench scale studies with actual shipboard testing.

Comment: (46)We included this section to ensure the reader that we have considered open ocean exchange and we are in agreement with the Coast Guard that it is ineffective, impractical, and in many cases dangerous.

- *What will be the design of these ports? How will their effects on organisms be established? How will their ability to provide a representative sample of the water flowing past be validated?*

b. Flowing water lines – seven total sampling points, [4] in the vertical tanks at 15, 30, 50 feet below the deck, and near the bottom of the tank; and [3] points in the horizontal section will be installed in one or more ballast tanks. The samples will be used for:
1. Bacterial enumeration
2. Phytoplankton identification and population densities
3. Chemical studies

c. To sample for zooplankton it will be necessary to use vertical plankton tows. (These organisms can avoid a sampling tube and therefore a non-representative sample is obtained.)

d. Niskin bottles (if necessary) will be used to complement the flowing sampling lines. However, we intend to minimize this sampling methodology in the interest of time and personnel requirements. Seasonal and tidal effects should be factored in if possible to maximize the number of different taxa sampled. Tidal effects will be dependent upon the vessel's operation schedule and will not be controlled experimentally.

- *d. Can you still maintain statistical validity of the experiment if you minimize the use of niskin bottles to complement the flow sampling lines? Please specify what level you mean when you say minimize.*

VII. Analyses

1. Ship Board

A portable laboratory on the ship near the ozone generator is planned. This will allow maximum flexibility and minimize the impact on the ship's operation. The analyses that would be conducted are:
a. Heterotrophic plate counts (if space is available)
b. Phytoplankton using chlorophyll a determination

- *Why –will chl a be correlated with concentration somehow?*

c. Zooplankton (if space is available)
d. Chemical characterization

2. Shore Based

a. Phytoplankton (flow cytometry)
b. 3-D Characterization of treated and untreated water

- *What does this mean? Does it include zooplankton?*
- *For zooplankton, how will viability be assessed? Movement? How will this procedure be standardized, and validated?*

Comment: (47)The specific design will be determined as part of our ongoing design engineering effort. However, we're extremely confident that the turbulent water flowing at 10,000 gpm in an 18" pipeline will ensure that a uniform sample is being obtained. See the following photographs of the ballast piping on the Prince William Sound. (http://www.nutech-o3.com/images/gallery/pages/pw2.htm)

Comment: (48)We will minimize the use of Niskin bottles because the process is labor intensive. We are only able to bring on board a limited number of crew in order to conduct sampling.
One can never be 100 % sure of representativeness of sampling lines but these are standard sampling techniques used in water quality analysis.

Comment: (49)Yes, with TRO. Standards will likely be expressed in terms of concentration of organisms. Here we are trying to obtain as much information as possible. We used direct counts previously; however, diatoms event though they are dead are counted – also some dinoflagellates. So if an organism is dead the chlorophyll a disappears rapidly and this is probably the best indication of effectiveness for determining the kill rate.

Comment: (50)Due to results obtained in recent experiments, we will not be doing shore based 3-D characterization. Forthcoming reports will contain these results.

Comment: (51)On our ship board experiments, zooplankton will be assessed as follows. A field of view at 25x magnification will be examined. Animal activity will be scored as follows: if animals are moving of their own accord or move away when probed with a fine needle (a 000 size insect pin mounted on a wooden stick), they will be scored as "alive;" if they are not mobile, but exhibit internal or external movement, they will be scored as "moribund;" and if they show no life, they will be scored as "dead." Successive fields of view will be examined until a total of 100 organisms are examined. In addition to these counts, qualitative observations will be made about which, if any, taxa appear to be more or less affected by the treatment.

VIII. Control and Monitoring

1. Incorporate both in-line measurements and individual sample analyses of the effluent (and the water in the ballast tank) to control the TRO.

2. Testing TRO similar to the use of disinfection monitoring used in drinking water.

IX. Scientific and Engineering Questions

1. What is the half-life and expected lifetime of the ozone/total residual oxidant (TRO) in ballast tanks?

2. What is the O_2 concentration in the ballast water after ozone injection and over the course of the return voyage to Valdez?

3. How well does the Venturi injection system and further contact in the ballast tanks accomplish the objective of ozone injection into the ballast water?

4. Are there any additional requirements with respect to the design of the ozone transfer/ contacting system?

5. What are the absolute numbers of the three groups of organisms remaining after various times in the treated ballast water?

X. Outputs

1. A comprehensive report that meets the requirements of Congress and NOAA.

- *This report should be peer-reviewed. The funding is not being provided for the type of basic research that is usually published in journals – it is being provided explicitly to develop/test an effective ballast water treatment system. The results are clearly intended to inform the development of regulations and policy. The government should not have to wait 1-2 years after the project is over for journal articles to make their way to press before finding out whether the findings are sound.*

Comment: (52)We agree. Our report to Congress and NOAA will not be peer reviewed as time does not allow for this.

2. A peer reviewed journal article on the photochemical fate of the TRO and potential environmental impact of discharge.

3. A peer reviewed journal article on full-scale ozone transfer studies aboard a ship.

4. One or more peer reviewed journal articles on the effect of TRO on potential exotic species transfer and bacterial inactivation.

5. A trade journal article on the use of ozone in marine applications, with the focus on system design, and operational safety issues.

6. A peer reviewed journal article on effluent toxicity, expanding the present database.

Page 2: [1] Comment **Mike Jennings** **4/28/2005 10:14 AM**

(1) We are preparing several papers that analyze our research from 2000 through 2005
1. Dr. Russell Herwig our senior biologist, from the University of Washington, is in charge of preparing the Report on the experimental studies conducted both on board the Tonsina and at the University of Washington's Marrowstone test facility.
2. Dr. William Cooper and Dr. Hans van Leeuwen, our principal scientific investigator and scientific advisor, are performing final review on a TRO – decay study.
3. Parametrix is preparing an Ecotoxicology paper from the first set of experiments on the Tonsina.
4. Dr. Herwig and Jake Perrins, of University of Washington, are preparing a Flow Cytometry analysis of the bacterial response to ozone involving Tonsina trials in CA and WA.
5. Dr. Herwig and Jake Perrins are preparing a paper on Mesocosm studies at Marrowstone Island looking at TRO decay micro phyto and zooplankton mortalities.
New studies are being conducted under the direction of Bill Cooper:
1. Photochemical degradation of TRO in surface waters
2. LC50 of three organisms to TRO

Page 2: [2] Comment **Mike Jennings** **4/25/2005 10:49 AM**

(2)Ozone injection by venturi is utilized in many drinking water treatment plants. Its effectiveness has been proven throughout the industry for many years. The focus of this research is to demonstrate the effectiveness of this technology in full scale testing on board a 125,000 DWT oil tanker during its regular operation.

Page 2: [3] Comment **Mike Jennings** **4/28/2005 9:27 AM**

(3) We agree, and note that these issues are covered as part of the design process, not the experimental protocol. Development of a detailed experimental design has not been presented before approval of the conceptual design to avoid wasting limited resources. Once approval is given, we intend to present and develop the detailed description of the experiments further. We feel this approach maximizes the productivity, eliminates duplication of effort and is in line with the concept of the "Cooperative Agreement" between Nutech O3, Inc. and NOAA.

Page 2: [4] Comment **Mike Jennings** **4/28/2005 9:30 AM**

(4)We agree and have covered these issues in design process. Prior to the ship owner allowing us to outfit the ship with this equipment, “whole ship” considerations will be covered as part of a detailed Hazardous Operations (HazOp) review. Present at this review are the ship owner (British Petroleum), ship operator (Alaska Tanker), system designer (Nutech/Netsco),US Coast Guard, and American Bureau of Shipping. No equipment is permitted to be installed until all conditions of the HazOp review are met.

Page 2: [5] Comment **Mike Jennings** **4/28/2005 10:13 AM**

(5)We have conducted extensive corrosion studies at the LaQue Corrosion Institute. See study at (http://www.nutech-o3.com/files/laque_corrosion.pdf). In addition, it should be pointed out that the ballast tanks are hardly exposed to molecular ozone considering the very short half-life of the ozone (less than or equal to five (5) seconds).

Page 2: [6] Comment **Mike Jennings** **4/27/2005 11:57 AM**

(7) We were looking to this being an iterative process and the formal design will be completed once the Panel approves the general approach. Our research will focus on the absolute number of organisms remaining in the treated water. We will not rely on percentage reductions, as an indicator of disinfestations success, as this is an invalid means of determining water quality after treatment since a 95% reduction in the total number of organisms per unit of water may still represent dangerous levels of contamination.

Page 2: [7] Comment **Mike Jennings** **4/28/2005 10:03 AM**

(9)Extensive discussions on the understanding of the system were included in our grant proposal and can also be found in our final report on the Tonsina experiments. (http://www.nutech-o3.com/files/2002june15_finalreport.pdf)